Long-Term Effects of Crude Oil Contamination and Bioremediation in a Soil Ecosystem

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Abstract: Analysis of samples taken from three experimental soil lysimeters demonstrated marked effects on the soil chemistry and on bacterial, fungal, nematode, and plant communities 3 years after the application of crude oil. The lysimeters are located at the Amoco Production Research Environmental Test Facility in Rogers County, Oklahoma, where they had been installed to evaluate the effectiveness of managed (application of fertilizer and water, one lysimeter) vs. unmanaged bioremediation (one lysimeter) of Michigan Silurian crude oil compared to one uncontaminated control lysimeter. Five 2-foot-long soil cores were extracted from each lysimeter, each divided into three sections, and the like sections were mixed together to form composited soil samples. All subsequent chemical and microbiological analyses were performed on these nine composited samples.

Substantial variation was found among the lysimeters for certain soil chemical characteristics [% moisture, pH, total Kjeldahl nitrogen (TKN), ammonia nitrogen (NH_4-N), phosphate phosphorus (PO_4-P), and sulfate (SO_4^{-2})]. The managed lysimeter had 10% the level of total petroleum hydrocarbons (TPH) of the unmanaged lysimeter. Assessment of the microbial community was performed for heterotrophic bacteria, fungi, and aromatic hydrocarbon-degrading bacteria (toluene, naphthalene, and phenanthrene) by dilution onto solid media. There was little difference in the number of heterotrophic bacteria, in contrast to counts of fungi, which were markedly higher in the contaminated lysimeters. Hydrocarbon-degrading bacteria were elevated in both oil-contaminated lysimeters. In terms of particular hydrocarbons as substrates, phenanthrene degraders were greater in number than naphthalene degraders, which in turn outnumbered toluene degraders. Levels of sulfate-reducing bacteria seem to have been stimulated by hydrocarbon degradation. Nematodes were extracted from soil samples, identified as to genus, and classified according to their mode of nutrition. All vegetation and roots were removed from each lysimeter after the soil samples were taken, representative plants were pressed for identification, and the dry weight of all plants (total biomass) for each lysimeter was determined. The plant species were predominantly those typically found in disturbed habitats. The greatest number of species was found in the control lysimeter, but the total biomass was highest in the managed lysimeter.

Keywords: crude oil, TPH, sulfate-reducing bacteria, nematodes, soil ecosystem, fungi, hydrocarbon.

Introduction

It is well established that most of the hydrocarbons of crude oils are amenable to biodegradation by microorganisms indigenous to soil provided sufficient aeration, moisture, and mineral nutrients are available. The ultimate objective of in situ bioremediation of petroleum-contaminated soil is to restore the economic and/or aesthetic value of the site. Clearly the long-term effects of the original contamination and the bioremediation process on the soil ecosystem are important determinants to the eventual postremediation use of the soil.

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In 1992, Amoco Production Company initiated a study of managed and unmanaged bioremediation of soil contaminated with Michigan Silurian crude oil. Three lysimeters were filled with topsoil; one was left uncontaminated as a control while the other two were contaminated with crude oil. Of the contaminated lysimeters, one received fertilizer and water (managed) and the other did not (unmanaged). The rates and extent of bioremediation in the contaminated lysimeters were then studied for 6 months. After the conclusion of this work the lysimeters were unattended and exposed to the elements.

The work presented in this paper was initiated 3 years after the original contamination of the lysimeters with the objective of evaluating the long-term effects of crude oil contamination and bioremediation on the soil ecosystem. This study consisted of a comparison of the three lysimeters on the basis of soil chemistry; bacterial, fungal, and nematode populations at different depths; and the plant life that had become established on the soil surface of each lysimeter.

Materials and Methods

Soil Lysimeters

The three soil lysimeters were set in place in 1992 at the Amoco Production Research Environmental Test Facility in Rogers County, Oklahoma. Each lysimeter measures 9.1 ft (2.8 m) by 9.1 ft (2.8 m) by 3 ft (0.91 m) [depth] and consists of a shallow, reinforced concrete container containing 9.2 yd^3 (7.1 m^3) of soil (Figure 1). Each lysimeter is drained by a pipe into a collection pond. The soil used to fill the lysimeters was collected from northwest Tulsa County and was representative of the loamy Okay series found in that area (Cole et al., 1977). This soil was not contaminated with salt or hydrocarbon. An analysis of this soil is presented in Table 1.

Table 2 shows the analytical results following application of 21 gallons of Michigan Silurian Reef crude oil to two of the lysimeters. The crude oil was applied by hand-spraying evenly over the surface and tilling to a depth of 1 foot (0.305 m) for an initial oil loading rate of 1.7% by weight. Fertilizer was added to one of the contaminated lysimeters (managed bioremediation) to provide nitrogen (0.73 kg, in the form of urea), phosphate (0.18 kg, as P_2O_5), and potassium (0.18 kg as K_2O). The managed lysimeter was watered as needed to maintain soil moisture at 80% of container capacity. TPH was monitored for 190 days by infrared absorbance (TPH-IR). By the end of this period, TPH decreased by 26% in the unmanaged lysimeter and by 88% in the managed lysimeter (Fisher and King, 1994).

Soil-Gas Measurements

The current project was initiated, 3 years after the original contamination with crude oil, with a soil-gas analysis in the control (C), oiled (O), and oiled and fertilized (OF) lysim-

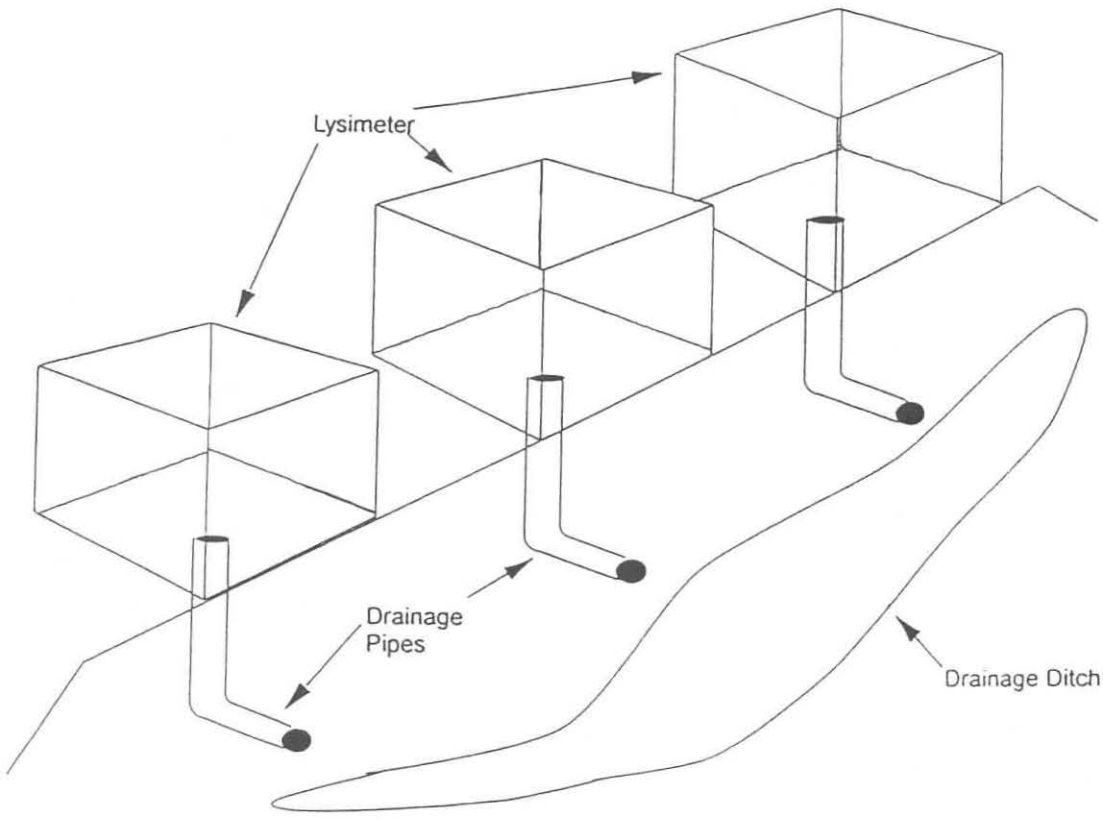

Figure 1. Schematic diagram of lysimeters used to study the long-term effects of crude oil contamination and bioremediation on soil ecology.

Table 1. Analysis of original lysimeter soil.

Soil Moisture (%)	9.6
pH	7.0
Saturated Paste Moisture (%)	26.4
CEC (meq/100 g)	4.8
NO_3-N (ppm)	0.3
PO_4-P (ppm)	30.2
EPTA K (ppm)	61.7
Soluble Cations (mg/L)	
Na	0.2
Ca	1.9
Mg	0.9
Exchangeable Sodium (%)	<1.0
TPH-IR (mg/kg)	17.5
TPH-GC (mg/kg)	4.1
Exchangeable Cations (meq/100 g)	
Na	<0.1
Ca	2.1
Mg	1.0

Source: Fisher and King (1994).

Table 2. Type analysis of Michigan silurian crude oil.

Carbon Number	Mole %
C4–C10	48.98
C11–C20	36.66
C21–C30	10.26
C31–C40	3.05
C41–C53	1.05

eters. Soil gases, including O_2, CO_2, volatile organic compounds (VOCs), and VOCs minus methane (VOC-Me), were measured 12 in (30.5 cm) below the surface at three (C, OF) or four (O) well-separated locations in each lysimeter. Soil vapors were sampled with an AMS soil-gas vapor probe (SGVP; Forestry Suppliers, Jackson, Mississippi). SGVP-dedicated sampling tips, perforated with vapor inlet holes, were driven 12 in (30.5 cm) into the subsurface. The SGVP drive tubes were removed leaving the vapor tip probe embedded at the desired sampling depth. A Teflon™ vapor tube, connected to the tip and extending to the surface, was used to sample soil gases near the tip. The VOCs in soil gases were measured using a GasTech TraceTechtor™ hydrocarbon analyzer with range settings of 100 ppm, 1,000 ppm, and 10,000 ppm. The analyzer was calibrated against a hexane calibration gas (4,350 ppm). Soil-gas concentrations of CO_2 and O_2 were measured using a GasTech model 32520X CO_2/O_2 analyzer. The CO_2 calibration was performed against the atmospheric CO_2 concentration (0.05%) and a 2.5% standard. The O_2 was calibrated using an atmospheric standard (20.9%). Both analyzers had an internal vacuum pump for sampling soil gases.

Soil Sampling

Soil core probes were made of 3-cm-inner diameter (I.D.) stainless-steel tubing. The inside surface of all probes was washed with methylene chloride to remove hydrocarbon residues. The probes were then sterilized by autoclaving at 300°C and 10 pounds per square inch gauge (psig) for 45 min. Soil cores were taken in a five-spot pattern from each lysimeter from the surface to 24 in (61 cm) below the surface. The probes were brought back to the laboratory to be processed immediately after collection. Using a tubing cutter, each probe was cut into three sections: surface to 3 in (7.6 cm) below surface (L1); 3 in (7.6 cm) below surface to 12 in (30.5 cm) below surface (L2); 12 in (30.5 cm) below surface to 24 in (61 cm) below surface (L3). Sterilized spoons were used to remove soil from each section of a probe. Soil samples were placed into pails that had been washed with methylene chloride and sterilized by autoclaving. Composite soil samples were made by mixing with a sterile spoon the five like sections (in terms of sample depth) from each lysimeter in the sterilized pails, giving a total of nine samples, three from each lysimeter. The composited soil samples were then subdivided for chemical, microbial, and nematode analysis. Five additional soil samples from the surface to 3 in (7.6 cm) below the surface were taken with a bulb planter from each lysimeter for nematode analysis. These samples were not composited and were stored at 4°C in plastic bags until shipped on ice that same day to Kansas State University for nematode analysis.

Soil Chemical Analysis

The pH, percent moisture, carbonate, bicarbonate, chloride, sulfate, sodium, calcium, magnesium, potassium, sodium adsorption ratio (SAR), cation exchange capacity (CEC), total Kjeldahl nitrogen (TKN), nitrate nitrogen (NO_3-N), ammonia nitrogen (NH_4-N), total phosphorus, available phosphate phosphorus (PO_4-P), and total carbon (%) were determined for each of the composited soil samples. The composited soil samples also were analyzed for total petroleum hydrocarbons both by gas chromatography (TPH-GC) and by infrared absorbance of a solvent extract (TPH-IR), and for benzene, toluene, ethylbenzene, and xylenes (BTEX). Samples were shipped by overnight delivery in completely filled glass jars with Teflon™-lined lids to Soil Analytical Services, Inc., College Station, Texas for analysis.

The composited soil samples also were tested by Microtox for residual toxicity. For each sample, 1.4 g of soil was extracted with 14 mL of Microtox Solid Phase Diluent. Soil and extract were separated by centrifugation and the toxicity of the extract was measured (Microtox M500 Manual, 1992).

Fungi

Sterilized water (9 mL) was added to 1 g of soil, mixed thoroughly by vortexing, then diluted and spread (three replicates) onto Malt Extract Medium (Difco Co.) containing 300 µg/mL of the antibacterial antibiotic streptomycin (Sigma Chemical Co.). The plates were incubated at room temperature for 2 weeks before colonies were counted.

Heterotrophic and Hydrocarbon-Degrading Bacteria

Sterilized water (10 mL) was added to 1 g of soil, mixed thoroughly by vortexing, then diluted and spread (three replicates) onto Plate Count Agar (PCA, Difco Co.) containing 40 µg/mL of the antifungal antibiotic cycloheximide (Sigma Chemical Co.), and onto a mineral salts agar with trace metals containing either toluene (TOL), naphthalene (NAP), or phenanthrene (PHE), to select for toluene-, naphthalene-, or phenanthrene-degrading bacteria, respectively. PCA media were incubated at room temperature for 48 hr before colonies were counted. Each of the three hydrocarbons was administered separately in the vapor phase as crystals (NAP, PHE) or as liquid on filter paper (TOL) placed on the inside of the petri plate lid. Each type of hydrocarbon medium was sealed separately in a Rubbermaid™ container, and incubated for 36 days at room temperature in an active chemical fume hood to prevent mixing of the vapors. Colonies of bacteria originally isolated on minimal medium containing toluene, naphthalene, or phenanthrene as a carbon source were transferred to plates of minimal medium containing one of the other two hydrocarbons, and were assayed for growth after incubation for 14 days.

Sulfate-Reducing Bacteria

Composited soil (3 g) was added to a bottle containing 9 mL of sulfate-reducing bacteria (SRB) medium (Bioindustrial Technologies Inc., Austin, TX). The soil and medium in the tube were thoroughly mixed by vortexing for 1 minute. A 1-mL sample was then withdrawn with a sterile needle and syringe and used to inoculate another 9-mL bottle of SRB medium. The procedure was repeated for a total of ten dilutions of the original soil sample, and was performed in triplicate for each of the nine composited samples. The tubes were incubated for 25 days then scored for growth and formation of a black precipitate (iron sulfide) indicating the presence of SRB. The most probable number (MPN) of SRB in the original samples was estimated from the characteristic number for a three-tube MPN (Rodina, 1972).

Nematodes

Five soil samples (approximately 100 cm^3 each) were collected from the surface of each lysimeter for nematode analysis. Samples (100 cm^3) of composited soil described above also were analyzed for nematodes. Nematodes were extracted from 100-cm^3 subsamples of soil using a modified Christie-Perry technique (Christie and Perry, 1951). The soil was suspended in 3.8 L of water, allowed to settle for 1 minute, and poured through a 38-µm pore sieve. The sievings were washed onto a single layer of Scotties brand tissue nested over a 10-cm-diameter pot filled with water. Following a 24-hr waiting period, the nematodes were concentrated by decanting and were counted. Nematodes were identified as to genus or subfamily using characters discernible at 100× magnification and assigned to trophic groups based on information summarized by Yeates et al. (1993).

The nematode community was described using the following diversity and maturity indices. The diversity of each nematode taxon and trophic group was estimated using the Shannon diversity index (H′),

$$H' = \exp(-\Sigma P_i (\ln P_i))$$

where P_i is the proportion of taxon or trophic group i in the total population (Shannon and Weaver, 1949). The maturity of the community was estimated using the maturity index (MI),

$$MI = (\Sigma V_i f_i)/n$$

where V_i is the colonizer-persister value assigned to taxon i, f_i is the frequency of taxon i in the sample, and n is the total nematode population density (Bongers, 1990).

Plants

All vegetation, including roots, was removed from each lysimeter after the soil samples had been taken. Individual plant specimens, representing each species present, were pressed for identification. The total biomass for each lysimeter was determined by air-drying and weighing the remainder of the vegetation.

Results and Discussion

Soil Gas

Results of soil-gas analyses (at 1 ft or 30.5 cm) are summarized in Table 3. Total VOCs were highest in the oiled and fertilized (OF) lysimeter compared to the oiled (O) and control (C) lysimeters. However, as shown in Table 3, methane comprised most of the VOCs in the soil gas of the OF lysimeter, suggesting that the fertilizer originally applied to this lysimeter has had a stimulating effect on methanogenesis in anaerobic zones. The substrates for methanogenesis could be products of incomplete aerobic degradation of the hydrocarbons. Elevated nonmethane VOCs in the O and OF lysimeters were accompanied by reduced oxygen (O_2) and elevated carbon dioxide (CO_2) concentrations indicative of aerobic biodegradation of petroleum hydrocarbons. The greater CO_2

Table 3. Soil-gas measurements.

Lysimeter	O_2 (%)	CO_2 (%)	VOC (ppm)	VOC–Me (ppm)
C	18–19	0.5–0.6	12–16.5	10–11.5
O	14.5–15.8	2.1–2.9	36–40.5, 119	30–37
OF	11.7–14	2.2–4.2	130–145	37–40

Soil gases were measured 12 in below the surface at three (C, OF) or four (O) well-separated locations in each lysimeter. The numbers span the range of values obtained.

ppm = parts per million; VOC = volatile organic compounds; VOC-Me = volatile organic compounds minus methane.

concentrations and lower O_2 concentrations in the OF lysimeter compared to the O lysimeter again suggest a stimulation of bioactivity 3 years after application of the fertilizer.

Hydrocarbon Analysis

As expected, no BTEX was detected in any composited soil samples. Results of analyses of composited soil samples for TPH are given in Table 4. For comparison, the TPH-IR levels in 12-in (30.5-cm) composited samples 190 days after oil application, as given by Fisher and King (1994), were 17.5 mg/kg (C); 8,440 to 16,277 mg/kg (O); and 1,511 to 2,169 mg/kg (OF). The data in Table 4 suggest that, although the soil in each oiled lysimeter was tilled to 1-ft depth after application, most of the oil remained near the surface. If the TPH-IR data in Table 4 for L1 and L2 are averaged over a 1-ft (30.5-cm) depth, the weighted averages are 5,400 mg/kg (O) and 1,060 mg/kg (OF). Therefore, in both the O and OF lysimeters, additional TPH-IR reductions have been realized since the 190-day analysis. It is interesting to note, however, that if the TPH-GC is taken to be the lighter fraction of the total hydrocarbons in the soil, the hydrocarbons in the oil and fertilized (OF) lysimeter have been significantly enriched for the heavier components compared to the oiled (O) lysimeter. The soil-gas analysis suggests that these heavier components or residual partially oxidized products of the original oil in place are still undergoing active aerobic biodegradation.

Soil Chemistry and Toxicity

Results of the chemical analysis of composited soil samples from the three lysimeters are given in Table 5. The following observations are made based on the comparative soil chemistry of the lysimeters:

1. The capacity of the soil for holding moisture correlated somewhat with TPH. Higher TPH-IR resulted in greater moisture retention in the soil.
2. There was a reduction in pH that correlated with greater hydrocarbon biodegradation. This is attributed to the production of organic acids as intermediates of hydrocarbon degradation, perhaps due to local oxygen limitations.
3. The oiled and fertilized (OF) lysimeter still contained elevated levels of NH_4-N, available PO_4-P, and TKN relative to the C and O lysimeters 3 years after application.
4. The OF lysimeter still contained significant levels of total carbon compared to the O lysimeter, out of proportion to the relative TPH levels. This suggests that high concentrations of partially degraded hydrocarbons that are not measured as TPH remain in the OF lysimeter and continue to fuel biological activity, as indicated by the soil-gas analysis.
5. Microtox toxicity (EC_{50} of 34 to 35%) was detected relative to the control only in L1 of the O lysimeter, suggesting a correlation with residual TPH.

Table 4. Total petroleum hydrocarbon analysis of composited soil samples.

Lysimeter	Level	TPH-IR (mg/kg)	TPH-GC (mg/kg)
C	L1	<10	<25
	L2	<10	<25
	L3	<10	<25
O	L1	17,237	6,000
	L2	1,452	249
	L3	30.7	60
OF	L1	1,633	84
	L2	863	75
	L3	<10	<25

C = control lysimeter; O = oiled lysimeter; OF = oil and fertilized lysimeter.

L1 = 0- to 3-in-depth composite; L2 = 3- to 12-in-depth composite; L3 = 12- to 24-in-depth composite.

Fungi

Figure 2 shows the mean concentration of viable fungi ($\log_{10}$)/g soil (dry weight) for each level of the three lysimeters. Fungi were most abundant in the uppermost level of all lysimeters, with higher concentrations in the O and OF lysimeters than in C, suggesting that one effect of crude oil contamination and subsequent bioremediation was to promote the growth of soil fungi. The positive effect seen in O and OF may be due partially to the crude oil acting as a carbon and energy source for fungi that can degrade oil, as fungi were abundant on media containing aromatic hydrocarbons. Also, the low pH in the O and OF lysimeters (Table 5) may have favored fungi at the expense of bacteria, as fungi are more tolerant of low pH.

Fungi isolated from L1 were identified to the genus level (Table 6). The lysimeters differed in which genus predominated: *Aspergillus* was isolated in very high numbers from OF, yeast from O, and nonsporulating fungi from C.

Bacteria

Mean Heterotrophic Bacteria. Aerobic heterotrophic bacteria were more abundant in the two uppermost levels of the lysimeters, but differed little among the lysimeters. The mean concentration of viable bacteria ($\log_{10}$)/g soil (dry weight) for each level of the three lysimeters was approximately 10^6. Crude oil contamination seemed to have relatively little effect after 3 years on densities of bacteria assayed on this medium, unlike its effect on soil fungi.

Hydrocarbon-Degrading Bacteria. Figures 3 through 5 give the mean concentration of viable bacteria ($\log_{10}$)/g soil (dry weight) capable of growth on a particular hydrocarbon for each level of the three lysimeters, after 36 days incubation. Toluene-degrading bacteria were present in all lysimeters and all levels at approximately the same low concentration (Figure 3). The concentration of naphthalene-degraders was

Table 5. Lysimeter soil chemistry.

Lysimeter	Level	pH	% Moisture	Soluble cations and anions (meq/L)							
				CO_3^{-2}	HCO_3^-	SO_4^{-2}	Cl^-	Na^+	Ca^{+2}	Mg^{+2}	K^+
C	L1	6.3	5.0	<0.1	2.0	0.3	0.7	0.1	1.2	0.5	0.1
	L2	6.6	11.5	<0.1	1.3	0.5	1.4	0.5	0.8	0.5	0.1
	L3	6.7	15.8	<0.1	1.2	0.5	0.8	0.4	0.8	0.5	0.1
O	L1	6.0	7.8	<0.1	0.6	0.7	0.2	0.1	1.1	0.4	<0.1
	L2	6.1	15.4	<0.1	1.0	0.4	0.2	0.1	0.9	0.4	<0.1
	L3	6.8	18.1	<0.1	2.4	0.3	0.2	0.1	1.6	0.5	<0.1
OF	L1	5.5	4.9	<0.1	0.9	0.4	0.4	0.1	1.0	0.4	0.2
	L2	5.8	12.5	<0.1	0.9	0.5	0.2	0.1	0.9	0.3	0.2
	L3	6.3	15.2	<0.1	1.4	0.4	0.3	0.2	1.0	0.4	0.1

Lysimeter	Level	SAR	CEC (meq/100 g)	TKN (mg/L)	NO_3-N (mg/L)	NH_4-N (mg/L)	Total P (mg/L)	Avail. PO_4-P (mg/L)	LECO Total C (%)
C	L1	0.1	4.1	155	<1.0	5.9	252	39.1	0.2
	L2	0.6	4.2	218	<1.0	3.1	198	39.0	0.2
	L3	0.5	4.0	112	<1.0	8.1	302	42.4	0.1
O	L1	0.1	4.7	1,160	<1.0	10.6	404	26.8	1.2
	L2	0.1	5.7	290	<1.0	9.7	301	36.2	0.3
	L3	0.1	5.8	154	<1.0	8.3	283	35.7	0.2
OF	L1	0.1	5.7	554	<1.0	14.7	284	69.0	0.6
	L2	0.1	5.4	359	<1.0	14.7	246	51.8	0.4
	L3	0.2	5.3	308	<1.0	12.9	276	33.8	0.3

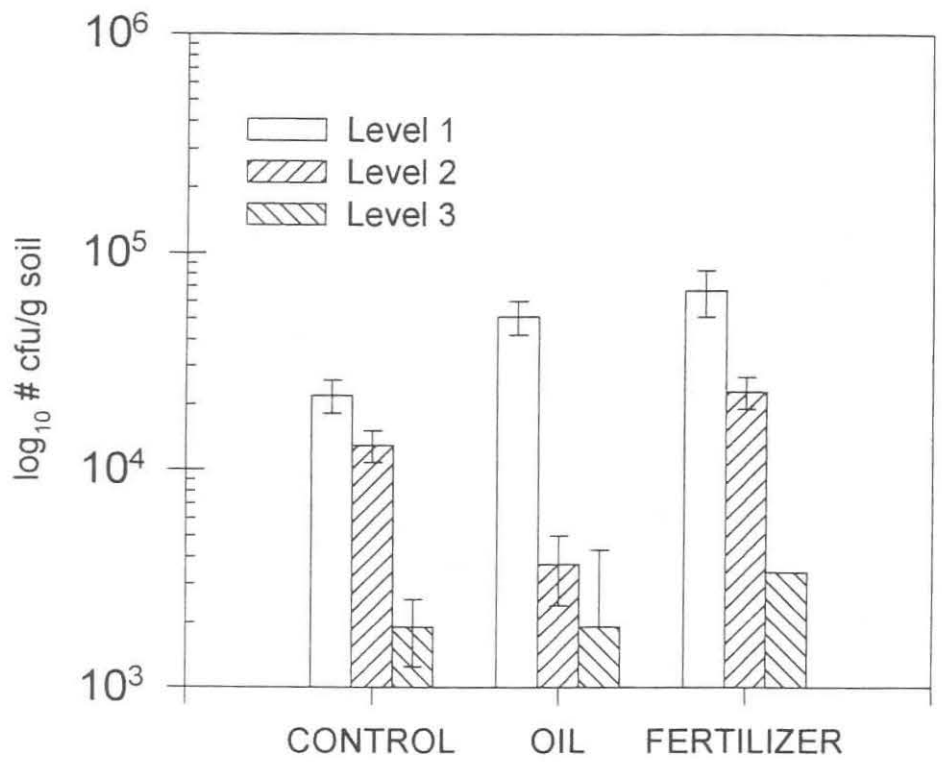

Figure 2. Concentration of viable fungi (log_{10})/g soil (dry weight) from each lysimeter. Values shown are the mean of three replicates; error bars are ±1 standard deviation (SD).

somewhat elevated in OL2 and OL3 above the corresponding levels in C, whereas the concentration in OF was elevated for all levels (Figure 4). Phenanthrene-degraders were much more abundant in OF and O than in C (Figure 5). The relative densities of these hydrocarbon-degraders (PHE>NAP>TOL) probably reflects the current level of these classes of compounds as the hydrocarbon is enriched for the heavier components. Fungi also were abundant on these plates, but they have not yet been examined.

Colonies of bacteria originally isolated on minimal medium containing toluene, naphthalene, or phenanthrene as a carbon source were transferred to minimal medium containing one of the other two hydrocarbons to investigate the multiple use of hydrocarbons by isolates. Results in Table 7 indicate that the application of oil appears to have selected for bacteria able to utilize more than one hydrocarbon. A higher proportion of colonies from the OF lysimeter than from the C lysimeter was able to grow on more than one aromatic hydrocarbon, regardless of which hydrocarbon was used in the original isolation. Notably, almost all colonies isolated on toluene from the OF lysimeter also grew on naphthalene and/or phenanthrene. A high proportion of colonies isolated on naphthalene from the O lysimeter also grew on toluene and/or phenanthrene.

Sulfate-Reducing Bacteria. The mean concentration of sulfate-reducing bacteria ($\log_{10}$)/g soil) in composited samples from each level of each lysimeter is shown in Figure 6. A higher concentration of SRB was found in L2 and L3 of each lysimeter than in L1, as expected for these anaerobic bacteria. Higher concentrations of SRB were found in each level in the lysimeters contaminated with crude oil than in the control. It appears that aerobic degradation of hydrocarbons in L1 has stimulated the growth of SRB especially in L3.

Nematodes

Total nematode densities were highest in the unfertilized, oiled (O) lysimeter (Table 8). Microbivores comprised 91% of the total population in the O lysimeter compared to 70% and 38% in the C and OF lysimeters, respectively. The microbivore trophic group includes nematode species that feed on unicellular eukaryotes in addition to bacteria (Yeates et al., 1993) and the observed increase in microbivore densities in the O lysimeter appears to reflect higher hydrocarbon concentrations (Table 4) and a concomitant increase in yeast densities (Table 6). In contrast, the fungivore trophic group, represented by hyphal-feeding species (Yeates et al., 1993) was not strongly affected by lysimeter treatment. The herbivore trophic group was a significant component of the nematode community only in the fertilized lysimeter (Table 8).

Diversity indices of nematode taxa and trophic groups were lowest in the O lysimeter, and highest in the C and OF lysimeters (Table 9). Contamination with crude oil negatively impacted the long-term diversity of the nematode community, whereas management of the system appeared to restore diversity to near uncontaminated levels. Maturity index (MI) values were similar for the C and O lysimeters but lower for the fertilized (OF) lysimeter (Table 9). The MI is based on a scale of 1 to 5, with 1 indicating colonizers and 5 indicating persisters. Fertilization typically results in a shift in the nematode community to colonizers and a subsequent decrease in MI (Bongers, 1990).

Plants

The C lysimeter contained the highest number of different species of plants with 11 identified species, one unidentifiable species, and various species of grass (Table 10). The OF lysimeter had 8 identified species and one unidentifiable specimen. The O lysimeter contained only five identifiable species and various grass species. The O lysimeter also had the lowest total dry plant biomass of the three (1.81 kg), but the OF lysimeter had a greater total dry plant biomass (3.45 kg) than did the C lysimeter (3.11 kg).

Conclusions

Three soil lysimeters, originally used to study the short-term degradation of crude oil in soil, have been reexamined 3 years after the application of oil. The application of crude oil and

Table 6. Genera of fungi isolated from lysimeter soil samples.

	Lysimeter		
Taxon	C	O	OF
Aspergillus	1,400 (600)	7,700 (3,700)	27,000 (17,000)
Cladosporium	—	—	690 (1,200)
Curvularia	—	350 (600)	350 (610)
Fusarium	—	—	1,700 (2,200)
Penicillium	350 (600)	3,500 (610)	3,800 (600)
Yeast	7,300 (4,800)	37,000 (3,200)	4,100 (4,100)
Nonsporulating	8,000 (4,210)	2,100 (1,800)	2,000 (4,900)
Unknown	1,400 (1,600)	—	—

Mean concentration (colony-forming units/g dry weight of soil) of fungi isolated from L1 samples, classified to the genus level. The numbers in parentheses represent one standard deviation.

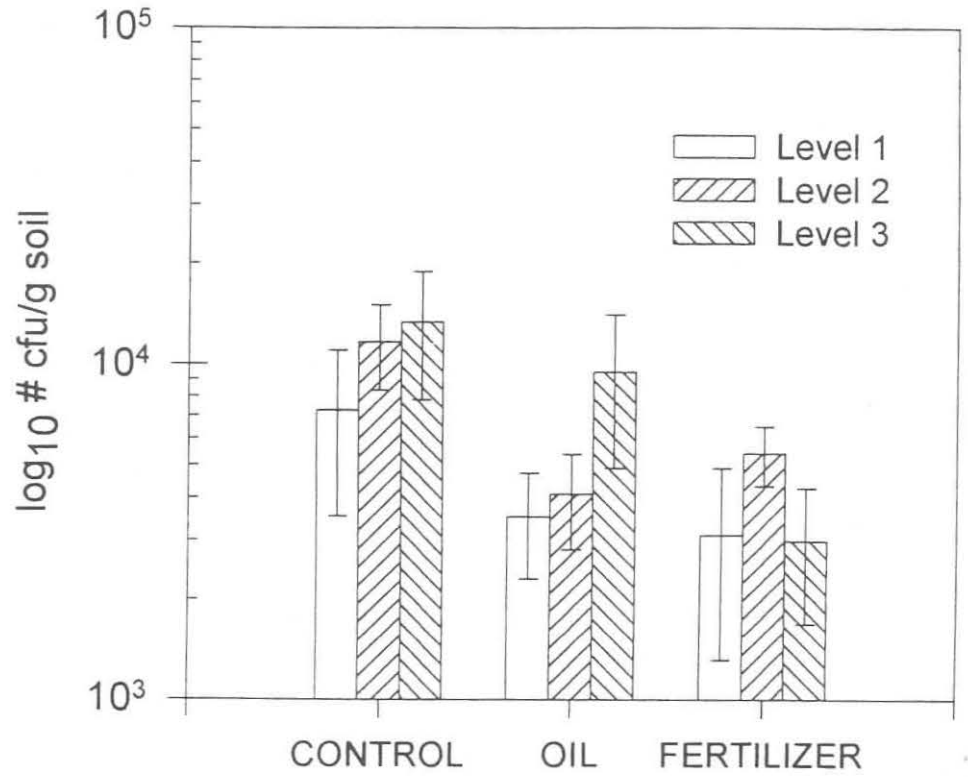

Figure 3. Concentration of viable toluene-degrading bacteria (log_{10})/g soil (dry weight) from each lysimeter. Values shown are the mean of three replicates; error bars are ±1 SD.

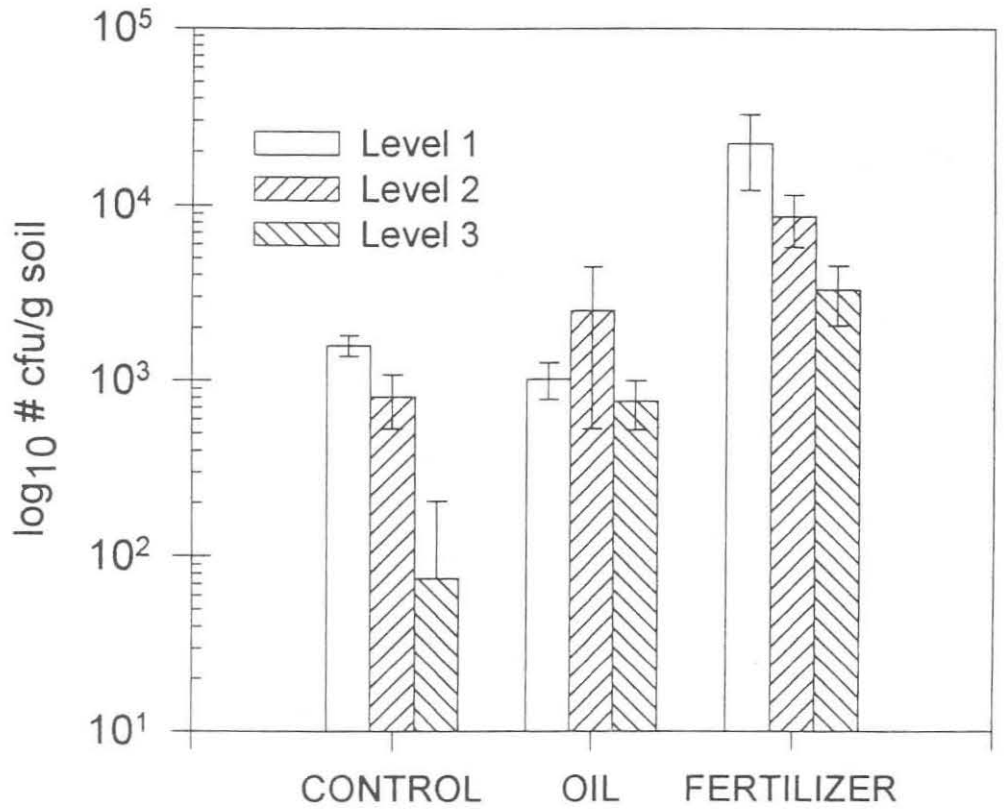

Figure 4. Concentration of viable naphthalene-degrading bacteria (log_{10})/g soil (dry weight) from each lysimeter. Values shown are the mean of three replicates; error bars are ±1 SD.

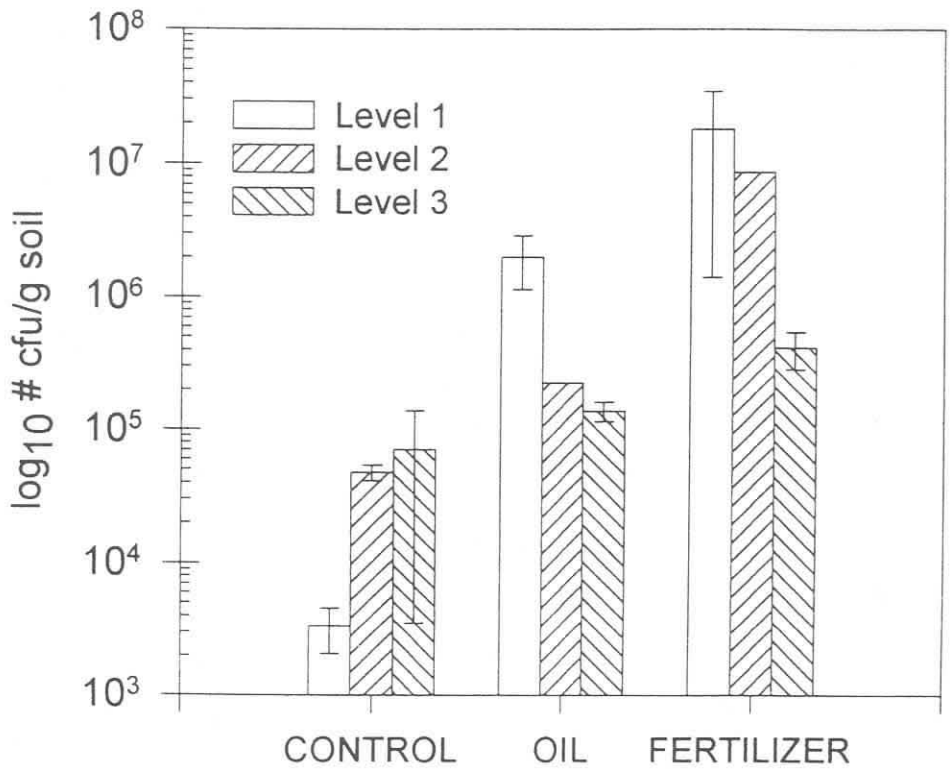

Figure 5. Concentration of viable phenanthrene-degrading bacteria (log_{10})/g soil (dry weight) from each lysimeter. Values shown are the mean of three replicates; error bars are ±1 SD.

Table 7. Use of multiple hydrocarbons by hydrocarbon-degrading bacteria.

Original hydrocarbon	Percent of growth on another hydrocarbon (# tested)		
	C	O	OF
Toluene	72.7% (22)	76.9% (26)	96.3% (27)
Naphthalene	60.9% (46)	88.6% (35)	73.7% (38)
Phenanthrene	27.3% (44)	22.5% (40)	35.0% (40)

Samples from all three levels were combined. The total numbers of colonies tested are enclosed in parentheses.

subsequent bioremediation (managed and unmanaged) have had marked effects on soil chemistry, microbial and nematode populations, and plant communities. Soil analysis showed that the oiled and fertilized (OF) lysimeter still contained elevated levels of NH_4-N, available PO_4-P, and TKN relative to the control (C) lysimeter and the lysimeter which received oil only (O). The application of fertilizer to a crude oil-contaminated lysimeter (managed bioremediation [OF]) compared to unmanaged bioremediation (O) has resulted after 3 years in:

- greater degradation of hydrocarbons (TPH),
- reduced Microtox toxicity,
- greater rates of oxygen consumption,
- increased methanogenesis,
- greater nematode diversity, and
- greater plant diversity and biomass.

However, although the OF lysimeter TPH was much reduced compared to the O lysimeter, the OF lysimeter still contained significantly higher levels of total carbon than the O or C lysimeters, suggesting high concentrations of partially degraded hydrocarbons. The soil-gas analyses strongly suggest that these compounds continue to fuel high levels of bioactivity. Both the O and OF lysimeters exhibited reduced pH and increased levels of fungi, SRB, and hydrocarbon degraders compared to the C lysimeter. Although managed bioremediation resulted in nematode diversity being restored

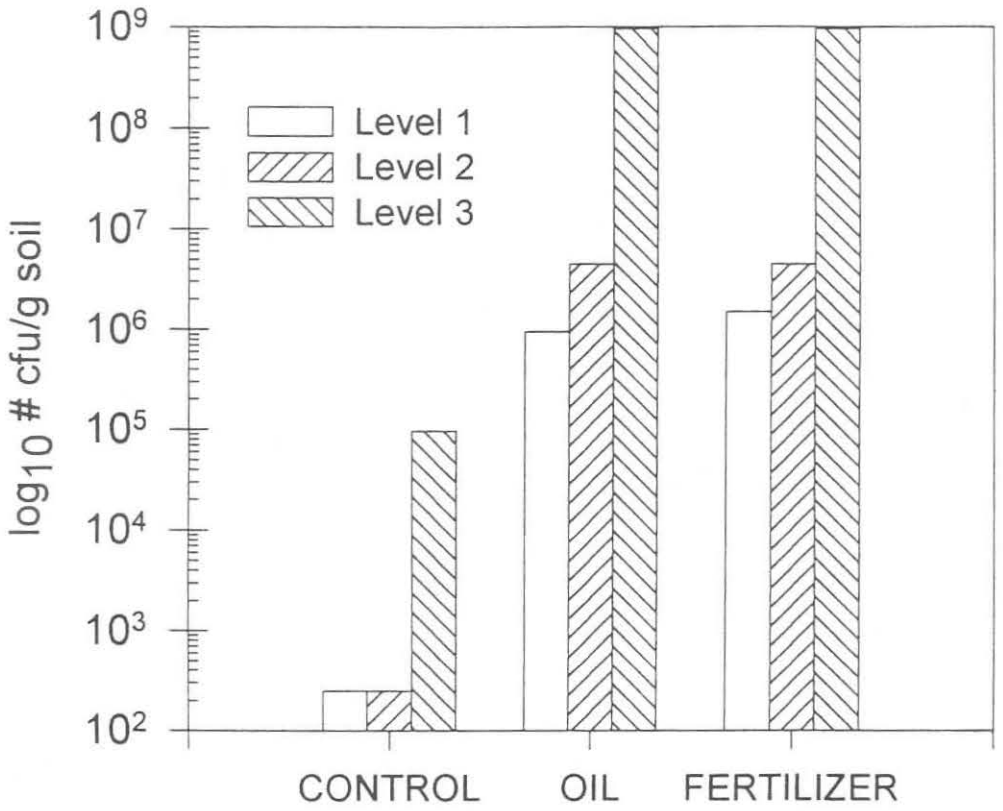

Figure 6. Concentration of viable sulfate-reducing bacteria ($\log_{10}$)/g soil (dry weight) from each lysimeter. Values shown are the mean of three replicates.

Table 8. Nutritional types of nematodes isolated from L1 of the C, O, and OF lysimeters.

	Average count (# of genera or subfamilies)		
Nutritional type	**C**	**O**	**OF**
Herbivores	ND	ND	362 (2)
Fungivores	140 (4)	120 (3)	72 (3)
Microbivores	360 (7)	1,220 (2)	264 (5)
Omnivore/Predators	12 (2)	ND	4 (1)

ND = none detected.

Average count is the average of five samples taken from level L1 in each lysimeter.

Table 9. Nematode diversity, trophic diversity, and maturity indices for the C, O, and OF lysimeters.

	Lysimeter		
Index	**C**	**O**	**OF**
Diversity	4.69 (0.91)	1.80 (0.41)	3.85 (0.47)
Trophic diversity	1.75 (0.19)	1.44 (0.15)	2.27 (0.23)
Maturity index	2.08 (0.05)	1.96 (0.04)	1.49 (0.07)

Mean and (standard error) of five soil cores collected from level L1 in each lysimeter.

Table 10. Plant species identified from lysimeter.

Plant species	Lysimeter		
	C	O	OF
Andropogon scoparius	No	No	Yes
Aristida oligantha	No	No	Yes
Bromus sp.*	Yes	No	No
Chenopodium album	No	No	Yes
Cirsium sp.*	Yes	Yes	No
Conyza canadensis	Yes	Yes	Yes
Croton sp.*	Yes	No	No
Echinochloa crusgalli	No	No	Yes
Grass spp.**	Yes	Yes	No
Hieracium sp.*	Yes	No	No
Krigia dandelion*	Yes	Yes	No
Lepidium virginicum	Yes	No	No
Oxalis stricta	Yes	Yes	Yes
Plantago aristata	Yes	No	No
Pyrrhopappus carolinianus	Yes	Yes	Yes
Rudbeckia hertia	Yes	No	No
Sorghum halapense	No	No	Yes
unidentifiable species (1)	Yes	No	Yes

*: unable to determine species. **: unable to determine genus.

Yes: species present. No: species not present.

to near the levels observed in the C lysimeter, plant diversity still suffered in the OF lysimeter after 3 years.

In summary, 3 years after crude oil contamination and subsequent managed bioremediation, the soil ecosystem clearly has been partially restored. However, more time and/or active intervention will be required for this disturbed ecosystem to return to normal as defined by the uncontaminated lysimeter.

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